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Effects of Nd:YAG Laser Irradiation on Morphometry and Lung Function in Elastase-Induced Emphysema in Rats

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Background & Objective: **Although thoracoscopic laser ablation therapy has been hailed as an effective surgical treatment for diffuse emphysema, no one has as yet made an in-depth study of the efficacy of this treatment. This investigation was undertaken to research the effects of laser pneumoplasty on an animal model of emphysema.**

Study Design /Materials and Methods: **Eight weeks after elastase treatment, the rats' left lungs were irradiated using contact Nd:YAG laser. Pulmonary function tests were performed 4 weeks after irradiation and the lungs were prepared for histologic examination.**

Results: **Dense fibrous scars beneath the pleura were observed at 4 weeks after irradiation. Although mean linear intercept values of irradiated lungs were not much lower than those in the non-irradiated elastase-treated group, laser irradiation caused a significant decrease in lung volume. While there was no significant difference in quasistatic compliance, elastic recoil pressure of the lung increased to control levels at total lung capacity volume.**

Conclusion: **We conclude that laser therapy does not cause normalization of compliance, or improvement in the deeper part of the emphysematous lung, but rather a peripheral volume reduction and "encasement effect" on the lungs as a result of fibrotic scars. Lasers Surg. Med. 23:204–212, 1998.**

Key words: rat; emphysema model; laser pneumoplasty; LVRS

INTRODUCTION

Emphysema is classified histopathologically according to what part of the acini it involves. In some types, the lung contains large bullae which bring about the collapse of adjacent lung structure. In other types, many minute bullae or spaces are distributed diffusely over the lung. Rogers reported that surgical removal of large bullae can result in improved airway conductance, and discussed the possible mechanism [1]. In "large bullae" type emphysema, resection works by relieving the surrounding areas from compression, while such an effect is rarely expected in the "diffuse" type.

In 1991, Wakabayashi [2] attempted to treat patients with diffuse bullous emphysema by thoracoscopic lung ablation using the CO_2 laser, which he reported was quite effective in improving pulmonary function. With the growing use of

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thoracoscopy, this technique has found wide favor, as documented by a series of reports in which its effectiveness was stressed [3–6]. However, in most of these reports, laser ablation was combined with resection of the bullous part of the lung. This combined use of irradiation and surgery has left the effect of the laser itself ambiguous. Moreover, at recent conferences on the surgical management of severe emphysema, some questions have been raised about the efficacy of laser therapy [7–9]. The problem is complicated by the lack of information about the pathological changes in the lung induced by this treatment, and of measures with which to evaluate its clinical effect in an objective way [10,11]. Therefore, we decided to undertake an experiment of an animal model to evaluate the effects of laser ablation and clarify the relationship between its structural and functional aspects. Panacinar emphysema was induced in rats by administering 4 biweekly intratracheal instillations of pancreatic elastase. Subsequently, the whole surface of the lung was evenly irradiated by contact Nd:YAG laser. After this treatment, changes in the lungs were studied histopathologically, morphometrically and by examination of lung function, and correlations among the results were analyzed.

MATERIALS AND METHODS

This experiment was approved by the Tohoku University School of Medicine Standing Committee on Animals.

Animals

Male Wistar rats weighing between 254 to 280 g were used. Prior to use in this experiment, the rats were kept in quarantine for 7 days to check their physical condition. To eliminate the effects of factors other than Nd:YAG laser irradiation, a total of 100 rats (not the number who survived and were analyzed but the total number of experimental rats) were divided into six groups as follows :

Non-emphysema groups, with endotracheal instillation of physiological saline

Group 1: instillation of saline only: 8 rats
Group 2: saline and thoracotomy: 8 rats
Group 3: saline, thoracotomy and laser irradiation: 12 rats

Emphysema groups, with endotracheal instillation of elastase

Group 4: instillation of elastase only: 28 rats
Group 5: elastase and thoracotomy: 8 rats
Group 6: elastase, thoracotomy and laser irradiation: 36 rats.

Creation of Emphysema by Enzyme Administration

The porcine pancreatic elastase (PPE; Owensville, Mo.,U.S.A. Elastin Products Inc.) used in this study had a specific activity of 75 IU/mg. The animals in Groups 4, 5, and 6 intratracheally received 50 IU/body of PPE immediately after it had been dissolved in 0.5 ml warm sterile physiologic saline. After being placed in a supine position, they actively inhaled the PPE solution in two or three breaths through a tracheal tube and then were gently shaken. To produce emphysematous changes severe enough and uniformly distributed in the lungs, instillation was repeated twice per week until the total dosage given amounted to 200 IU. No animals died during the period of instillation with either PPE or saline.

Thoracotomy and Laser Irradiation

The enlargement process of air spaces was near completion at 8 weeks after the last instillation of PPE or sterile saline, so this was when the rats underwent thoracotomy under general anesthesia with 30 to 40 mg/kg body weight (bw) of sodium pentobarbital given intraperitoneally with the addition of methylprednisolone sodium succinate (20 mg/kg bw). The anesthetized rat was intubated, connected to a respirator, placed in the right lateral position, and a left anterolateral thoracotomy was made through the 5th intercostal space, whether laser irradiation was performed or not. After thoracotomy, the left lung was irradiated as evenly as possible at each site up and down the visceral pleura for an average total exposure time of 10 minutes. A contact Nd:YAG laser (Laserscope SLT Laser, SLT Japan, Tokyo) operating at 1,064 nm and at a power of 5 watts, was applied for 0.5 to 1 seconds with calibration of contact tip prior to exposure, a procedure corresponding to an energy transfer of 0.62 to 1.2 J/cm^2.

Pulmonary Function Tests

At 4 weeks after laser irradiation, the scar tissue was completely hardened, so at this time, the rats were subjected to measurements of total respiratory system compliance (Ctr), dynamic compliance (Cdyn), functional residual capacity

(FRC), total lung capacity (TLC), and residual volume (RV).

All of the function tests were carried out using the whole-body plethysmographic method according to Lai et al. [12–14] under general anesthesia with sodium pentobarbital, 30 to 45 mg/kg bw, given intraperitoneally. Pressure changes in the body box were detected by a sensitive transducer (Sensym, SCLK-004), and both the airway and esophageal pressure were measured by another transducer (OMEDA, SCK-60). Pressure-volume (PV) curves of the total respiratory system were obtained using slow inflation and deflation by repaired syringe pump. TLC was defined as the lung volume at an airway opening pressure (Pao) of 25cmH_2O. The volume at Pao –20 cmH_2O was defined as RV. With similar studies, it has been demonstrated that chest-opened lung compliance did not differ from chest-intact lung compliance in control rats [12] and Pes drained by the esophageal catheter of the paralyzed rats could not be measured accurately after they had undergone thoracotomy in this experiment. Therefore, we treated compliance of the total respiratory system as the most confident parameter of elastic recoil function. Since hyperexpansion of the lung affects chest wall pressure, this may be an important factor in the compliance of the total respiratory system in the TLC region.

Microscopy and Morphometry

Animals were sacrificed after lung function tests were completed. Immediately after an intravenous injection of KCl, the lungs were fixed in situ by intratracheally instilling 10% buffered formalin according to Weibel [15]. After 24 hours, the intratracheal catheter was removed and the trachea tied up. The lungs were removed from the thorax as a single block and again fixed for another 24 hours in 10% buffered formalin. The lungs were then cut into frontal slices at a thickness of 3 mm and embedded in ordinary paraffin.

The progression of emphysematous changes was determined by the mean linear intercept (MLI), a method proposed by Dunnill et al. [16]. Using a microscope equipped with a linear eyepiece, a lung section was observed at a magnification of ×100 so that the sectional picture might randomly be overlaid with the line. The length of the sampling was designed so that the alveolar walls were intersected at more than 1,000 points.

Data Analysis

The unpaired 2-tailed *t*-test was used to determine whether the statistical differences between the groups were significant. The differences between treatments were sought by one-way or non-parametrical analysis for variance, and homogeneity was demonstrated. We used Pearson correlations to quantify the linear relationships between the two indices of pulmonary function tests and morphometrical data.

RESULTS

Changes in Elastase-Treated Lungs

At the beginning of our study, elastase was instilled into the airway and caused the lungs to develop enlarged terminal air spaces, resulting in panacinar emphysema combined with some distal acinar characteristics. This condition (Fig.1A,B) induced a significant MLI increase in comparison with the control group (at 2 weeks after instillation: 112 ± 2.6, at 3 weeks, 116 ± 2.5, at 6 weeks, 117 ± 4.1, at 8 weeks, 120 ± 1.5 μm : Mean ± SEM). Emphysematous changes were almost completely homogeneous in distribution. No widening of air spaces larger than 2 mm in diameter on the surface was confirmed to emerge. “Bullous” spaces, in the strict sense of the word, were not detected even in the subpleural zone; however, rather severe emphysematous lesions were observed in the upper and lower apexes. MLI of the subpleural region (1000 μm from pleura) vs. the deeper part was 1.15 : 1 in the control and 1.23 : 1 at 2 weeks after elastase treatment, 1.21 : 1 at 4 weeks, 1.28 : 1 at 6 weeks, and 1.3 : 1 at 8 weeks. Pulmonary function tests indicated significant increases in volume and compliance with PPE when compared with the saline group.

Mortality Rate for Laser Irradiation

Laser irradiation was so severe for rats that survival rate was 46% in the elastase-treated group and 47% in the control group. They died from respiratory failure problems such as respiratory distress syndrome, massive early air leakage, or hemorrhage from the pleura, but late air leaks did not occur, so no animals died from pneumothoraces.

Histological Observation After Laser Irradiation

Laser irradiation caused the peripheral lung tissue (745 ± 18 mm from pleura: Mean ± SEM) to become inflamed, and scarring continued to be observed over the 4 week period of our experiment (Fig. 2A–D). Deeper sections of lung tissue were encased in the dense newborn scar.

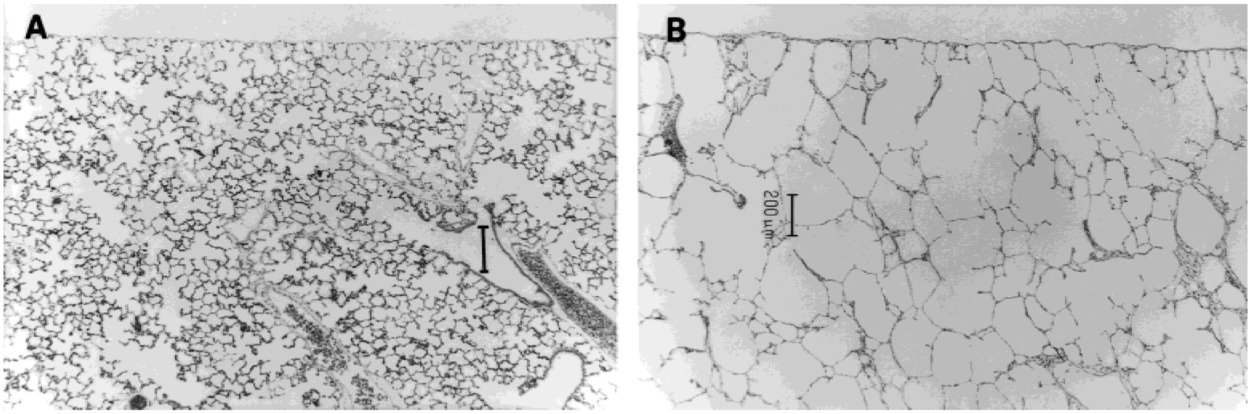

Fig. 1. Photomicrographs of control (**A**) and elastase-treated rat lungs (**B**). 12 Weeks after treatment, foci of dilated and distorted air spaces and some bronchi with monocyte infiltration were observed. Scale bar: 200 μm [A,B: Hematoxylin-eosin (HE) ×30].

Morphometrical Data

MLI values of laser-irradiated lungs vs. non-irradiated lungs are summarized in Table 1. The results show a slight decrease in MLI of the irradiated group when compared with the non-irradiated group of elastase-treated rats (Group 4 vs. Group 6) (113.6 ± 3.2 vs. 122.1 ± 2.5 μm : Mean ± SEM). When laser was applied to the left lungs, there was no significant difference between the left and right lungs (right lung refers to the right lobe and diaphragmatic lobe) in both control and elastase- treated rats (Group 3; left : 81.8 ± 2.3, right: 84.2 ± 2.5 mm, Group 6; left: 113.6 ± 3.2, right: 117.4± 2.3 mm : Mean ± SEM). No significant difference was revealed in the elastase-treated right lung between non-irradiated rats (Group 4) and the irradiated rats (Group 6) (123.2 ± 1.6 vs. 117.4 ± 2.3 μm : Mean ± SEM).

Physiological Data (Pulmonary Function Tests)

There were no significant differences between the rats that underwent thoracotomy without irradiation and those that did not undergo thoracotomy in both the control and elastase-treated groups in pulmonary function tests (Tables 2 and 3). Tables 2 and 3 summarize the pulmonary function tests in rats with normal lungs (Groups 1, 2, 3) and those with elastase-treated lungs (Groups 4, 5, 6). FRC and RV, or TLC of animals irradiated with laser were lower than those of non-irradiated animals in both the control and the elastase-treated groups (7.86 ± 0.76 vs. 5.63 ± 0.28, 5.0 ± 0.72 vs. 2.55 ± 0.26, 21.8 ± 1.8 vs. 18.1 ± 0.55 ml: Mean ± SEM). In the same manner, the RV/TLC decreased dramatically after laser irradiation (0.22 ± 0.02 vs. 0.14 ± 0.01: Mean ± SEM). Quasi-static compliance of the total respiratory system, and dynamic compliance of Group 6 were lower than that of Group 3, but no significance was revealed. Maximum airflow in spontaneous breathing of both the control and elastase-treated groups did not differ between the irradiated and non-irradiated rats. Figure 3 reveals the deflation PV curve of the total respiratory system pressure shown in absolute values of lung volume. The PV curve for the irradiated rats, located between that of the control group and the elastase-treated group, more closely resembles the latter group near the FRC region. In Figure 4, which shows the deflation PV curve for % TLC, the curve for Group 6 is also between that of Groups 1 and 5, and Group 4; the total respiratory system pressure is somewhat larger than the elastase-treated group at the same % (the PV curve has shifted to the right). The curve for Group 6 overlaps the slope of the control group as it approaches the TLC region, while its slope is parallel to that of the elastase-treated group near the FRC region.

DISCUSSION

Although it has been 6 years since Wakabayashi's first report came out, the direct effects of laser pneumoplasty are still unclear. Although the laser is useful for shrinking superficial lung tissue, no one has yet reported whether it could penetrate to the deeper part of the emphysematous lung, or if fibrous scars resulting from irradiation could actually improve pulmonary function through decreased lung compliance. Thus, we decided to look at the histopathological

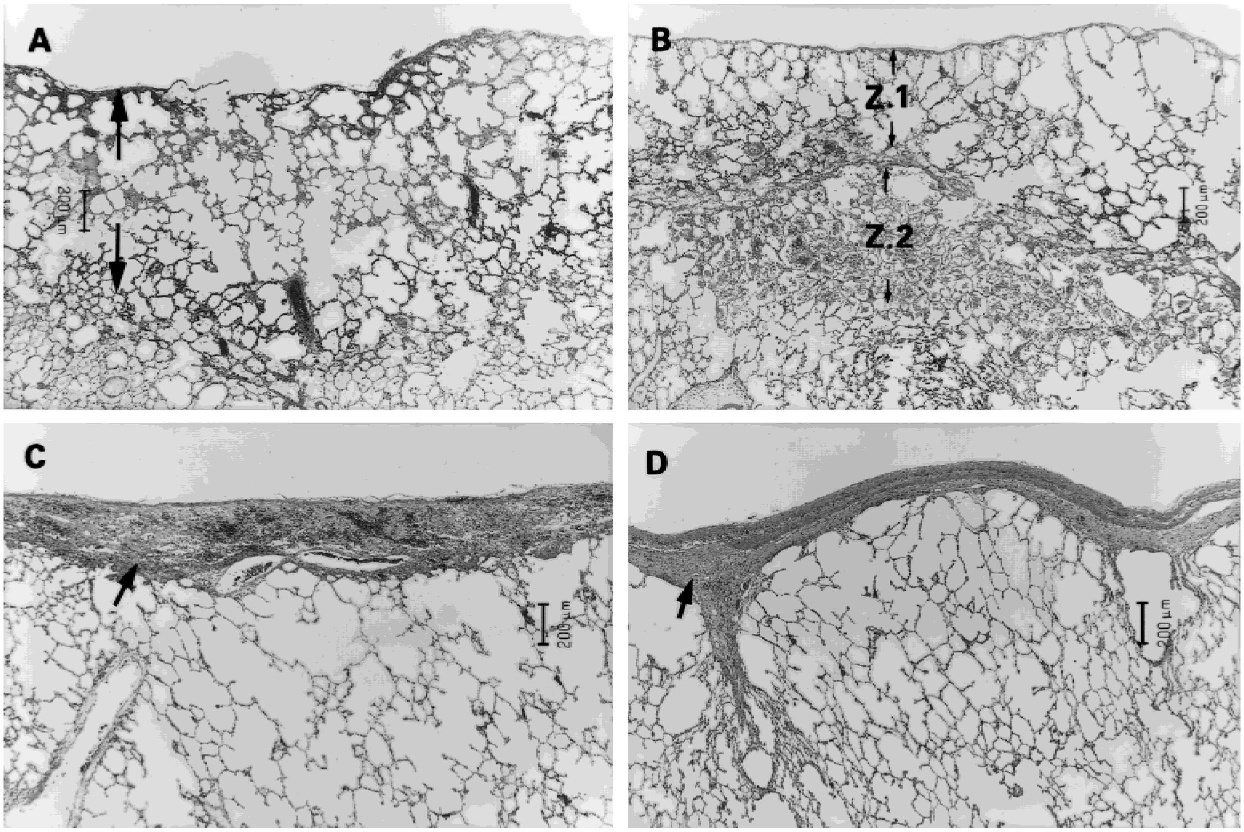

Fig. 2. Microscopic appearances of the lung after Nd:YAG laser irradiation. **A:** At 1 hour after laser irradiation, severe edema and coagulated alveolae were seen in the peripheral zone to about 1000 μm from pleura (arrow). Scale bar: 200 μm (A: HE ×30). **B:** At 3 days after Nd:YAG laser irradiation. The alveolae became necrotic in the subpleural zone and were clearly bordered by a demarcation line caused by severe hemorrhage (Zone 1: indicated as Z.1). Reactive hyperemic alveolar walls surrounded the inflammatory cells and numerous macrophages below the demarcation line (Zone 2: indicated as Z.2). Scale bar: 200 μm (B: HE ×25). **C:** At 1 week after laser irradiation, alveolar walls in Zone 1 began to collapse, while containing some inflammatory cells and erythrocytes (arrow). Scale bar: 200 μm (C: HE ×30). **D:** At 4 weeks after Nd:YAG laser irradiation, flat, well-defined, hard-looking layers of marked fibrous scar tissue were observed (arrow). Scale bar: 200 μm (D: HE ×30).

TABLE 1. Morphometric Results (Mean Linear Intercept)*

Group	Left MLI (Irradiated Side μ m)	Right MLI (Non-irradiated Side μ m)
Control n = 7	81.3 ± 0.4	83.7 ± 1.3
Cont.+Las. n = 5	81.8 ± 2.3	84.2 ± 2.5
Ela.-treated n = 8	122.1 ± 2.5	123.2 ± 1.6
Ela.-treated+Las. n = 12	113.7 ± 3.2	117.3 ± 2.3

*There is no significant difference in MLI between rats that underwent laser irradiation and those that did not in both control and elastase-treated groups. (P <0.05). The results are expressed as Mean ± SEM.

changes of lung parenchyma after irradiation in an animal model, and then investigate the morphometrical aspects and lung functions to identify the effects of Nd:YAG laser therapy on emphysema. In the following, we review the effects of laser pneumoplasty, i.e. compliance, morphometry, volume reduction and airflow through analysis of histopathological changes on peripheral lung tissue.

In our study, the effects of Nd:YAG laser characteristically showed penetration to the deeper sections of the peripheral zone (745 ± 18 μm from pleura), causing transpiration effects in the alveolar walls beneath the pleurae. In the chronic phase, the thick fibrous scar tissue in the subpleural part was revealed to be an accumulation of the necrotic alveoli that had become inflamed immediately after irradiation. What we observed on the peripheral portion was similar to the process of healing. In the past, there have been some reports about the pathological examination of lung parenchyma after laser irradiation [17–19]. Although both Cole and Brenner viewed peripheral changes as a side effect of lung injury after irradiation, we hypothesized that dense ne-

TABLE 2. Body Weight, Respiratory Variables in the Control Group

Group	n	Body weight (g)	FRC (ml)	RV (ml)	TLC (ml)	RV /TLC	Ctr (ml/cmH_2O)	Cdyn (ml/cmH_2O)	Max. flow in spont. breath. (ml/sec.)
Control	7	401 ± 6.6	3.78 ± 0.20*	1.8 ± 0.10*	13.8 ± 0.69*	0.13 ± 0.014*	0.58 ± 0.03	0.37 ± 0.028	15.6 ± 1.02
Control + Las.	5	381 ± 10	2.21 ± 0.21*	0.43 ± 0.11*	11.7 ± 0.53*	0.03 ± 0.008*	0.55 ± 0.03	0.33 ± 0.026	13.9 ± 1.06
Control + Thora.	5	402 ± 9.8	4.14 ± 0.34	2.18 ± 0.20	15.4 ± 0.28	0.14 ± 0.010	0.53 ± 0.03	0.40 ± 0.026	12.8 ± 0.31

*P <0.05. The results are expressed as Mean ± SEM.
FRC : Functional residual capacity; RV : Residual volume; TLC : Total lung capacity; Cdyn : Dynamic compliance; Ctr : Quasistatic Compliance of Total Respiratory System; Max. flow in spont. breath. : Maximum airflow of spontaneous breathing.

TABLE 3. Body Weight, Respiratory Variables in the Elastase-Treated Group

Group	n	Body weight (g)	FRC (ml)	RV (ml)	TLC (ml)	RV /TLC	Ctr (ml/cmH_2O)	Cdyn (ml/cmH_2O)	Max. flow in spont. breath. (ml/sec.)
Elastase-treated	8	411 ± 5.5	7.86 ± 0.72*	5.0 ± 0.72*	21.8 ± 1.4*	0.22 ± 0.019*	0.90 ± 0.040	0.56 ± 0.04	11.8 ± 0.62
Elastase-treated + Laser	12	417 ± 3.9	5.63 ± 0.28*	2.55 ± 0.26*	18.1 ± 0.55*	0.14 ± 0.012*	0.80 ± 0.028	0.48 ± 0.05	11.7 ± 0.33
Elastase-treated + Thora.	6	392 ± 6.3	8.33 ± 0.34	5.34 ± 0.16	19.7 ± 0.89	0.265 ± 0.083	0.83 ± 0.060	0.65 ± 0.09	11.8 ± 0.55

*P <0.05. The results are expressed as Mean ± SEM.
FRC : Functional residual capacity; RV : Residual volume; TLC : Total lung capacity; Cdyn : Dynamic compliance; Ctr : Quasistatic Compliance of Total Respiratory System; Max. flow in spont. breath. : Maximum airflow of spontaneous breathing.

crotic peripheral alveoli was not merely a side effect, but one of the most conspicuous effects that could bring on normalization of lung compliance. However, the results of our investigation did not appear to confirm this assumption. Results indicated that the quasi-static Total Respiratory Compliance was less responsive to laser irradiation than we had expected. The shape and slope of the PV curve near the FRC of the irradiated rats were not very different from that of the elastase-treated rats (Fig.3); also, Figure 5 showed that MLI did not have a positive correlation with quasi-static Total Respiratory Compliance, while FRC did. Therefore, the volume reduction effect (right shift of the PV curve) plays a relatively important role in changes of compliance. Nevertheless, elastic recoil pressure of lung increased to control group levels near the TLC volume as shown in Figure 4, because when lung volume increased to TLC or the lung became hyperinflated, lung elastic recoil pressure also increased due to compression from the subpleural scar tissue. This dense fibrous scar encased the deeper emphysematous lung tissue, and this condition we refer to as the "encasement effect". This condition, in turn, caused the elastic recoil pressure to in-

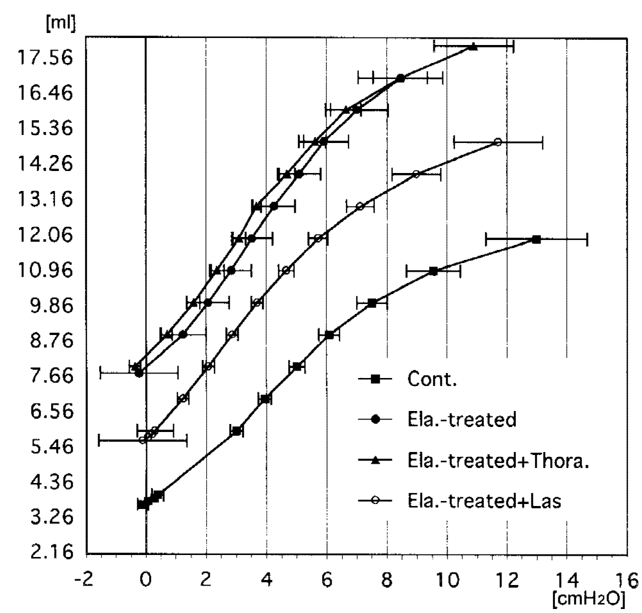

Fig. 3. Deflation pressure-volume (PV) curves with mean volumes plotted against total respiratory system pressure (Mean ± SEM). There is no difference in the slope of the curve between elastase-treated+ laser group and elastase-treated group.

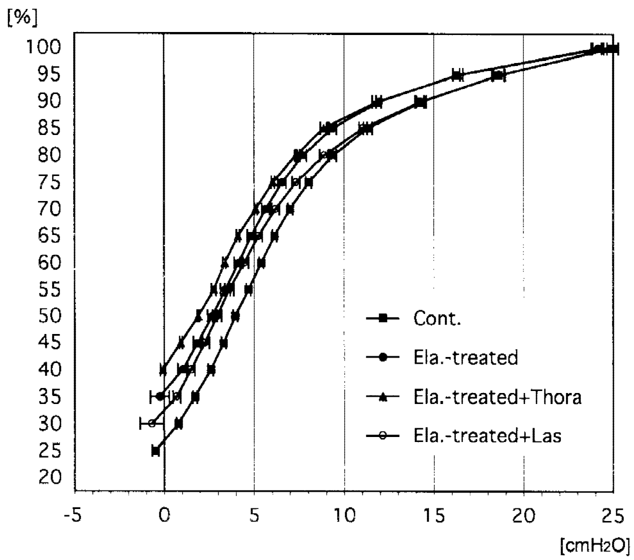

Fig. 4. Deflation pressure-volume (PV) curves with volume plotted as % total lung capacity (TLC) vs. total respiratory system pressure (Mean ± SEM). The total respiratory system pressure of laser rats is somewhat larger than the elastase-treated group at the same % TLC. The curve for group 6 overlaps the slope of the control group as it approaches the TLC region, while its slope is parallel with that of the elastase-treated group near the functional residual capacity (FRC) region.

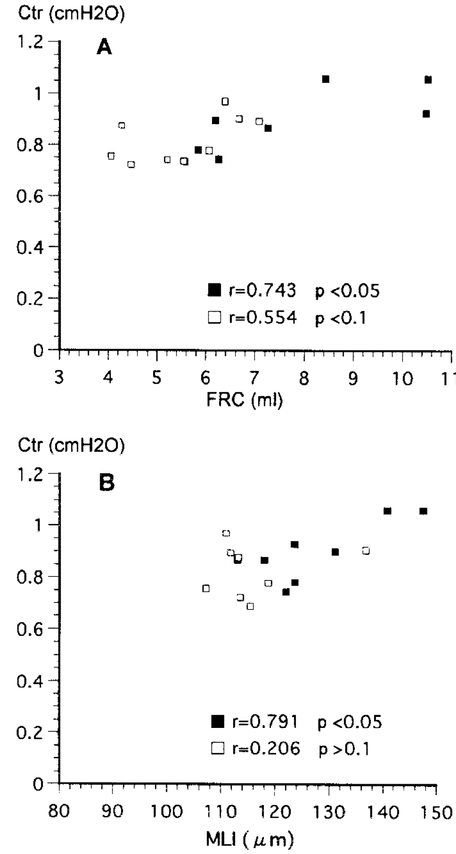

Fig. 5. Relationship between quasistatic total respiratory system compliance (Ctr) and morphometric indices (MLI), and FRC. MLI did not have a positive correlation with Total Respiratory Compliance, and FRC had a positive correlation with Ctr. **A:** Ctr is plotted against the FRC. **B:** Ctr is plotted against the MLI. Open squares: Elastase-treated+laser animals. Closed squares: Elastase-treated animals.

crease, making this the most striking consequence of laser pneumoplasty. We realized that one unresolved question still remains i.e. that the reduced RV is much larger than the reduction in TLC, and similar findings are seen in human cases. The newborn scar tissue must affect the inspiratory reserve volume (TLC) more than the RV. We suppose that the increased volume of peripheral part along with emphysematous change was much greater than the deeper area (p. 9, lines 13–15), and the air in the terminal air spaces was likely to become trapped gas, therefore, this could be the most probable cause of increased RV. Subpleural loss of volume by laser irradiation brings out a predominant reduction of the RV. Also, in emphysema rats, chest wall pressure at the TLC region might increase the Pao so that TLC could actually be much higher than the data suggests. This, in turn, may result in reduced TLC that is larger than our data reveals. It is difficult to clarify this dilemma because of the small models involved.

Our calculation of morphometrical values showed that laser irradiation produced little significant improvement on the MLI in the elastase-treated group, so we deduced that this treatment was not as effective for deeper emphysematous changes as we had originally expected.

We next looked at volume reduction. Previous clinical studies have reported a sizable decrease in lung volume or increase in forced vital capacity (FVC) after surgery, although there was no clear demonstration of how the improvement occurred. In our experiment, it was demonstrated that lung volume was reduced after laser irradiation. If laser irradiation had no discernible effect on the deeper part of the lasered lung or opposite lung tissue, we hypothesized that necrosis of the lasered alveolar tissue would produce this volume reduction. We calculated the radius of elastase-treated left lungs with the radius of left lungs after laser irradiation. The difference in radii between the two is about 1100 μm. As a result, this distance is only a little larger than the actual depth of the inflamed zone after Nd:YAG laser irradiation (745 ± 18 μm) as observed in our histological examination. Since subpleural volume is

usually larger than the deeper part of the lung because of more air spaces in the peripheral part, we concluded that the reduced volume in this study was almost equal to that of the peripheral alveolar parts that had become necrotic. As another factor affecting volume diminution, we suspected that the alveolar deformities near the scar tissue was as important as the decrease in MLI value.

Volume reduction surgery has been receiving a great deal of attention as an effective treatment for Chronic Obstructive Pulmonary Disease (COPD) patients [20]. With the prevalence of this treatment, some recent reports explained how pulmonary function improved following lung-reduction surgery for emphysema [21]. More investigators are accepting the theory that the resection of lung volume may result in a rightward shift of the lung PV curve, thereby increasing transmission of driving and elastic recoil pressure of the remaining lung tissue, and improving airway conductance [22–25]. It is still not known, however, how the narrowed airway affects the pathology or physiology of emphysema [26]. We assumed that laser irradiation on the surface area would play only a minor role, since there are few airways in the subpleural area which affect conductance.

In our experiment, although the surface area irradiated was much larger than is usually done clinically, the decline of volume we observed was only about 10% of TLC in rats' lungs with a radius of 1.8 cm. In previous clinical trials, with TLC at 8000 ml, contact Nd:YAG laser penetrated human bullous peripheral lung tissue to a depth of about 300–500 μm. Therefore, we supposed that the encasement effect and reduction value of lung volume would be minimal in humans since only small portions of the lung surface would be irradiated. Also, with bilateral volume reduction surgery, about 20–40% of each lung volume could be resected with staplers. We concluded that the stapler method is more useful than laser pneumoplasty in volume reduction therapy.

In argument for the improvement of emphysema, evidence of the release of airflow obstruction is paramount. In this experiment, we could not see any improvement in airflow obstruction. Airway dynamics of the lung for low lung volume such as in spontaneous breathing will probably not be affected by the "encasement effect". Since the only measures that were used to detect airflow obstruction were dynamic compliance and maximum airflow of spontaneous breathing, further investigation of forced expiratory airflow studies should be conducted [27,28].

REFERENCES

1. Rogers RM, DuBois AB, Blakemore WS. Effect of removal of bullae on airway conductance and conductance volume ratios. J Clinc Invest. 1968; 47:2569–2579.
2. Wakabayashi A, Brenner M, Kayaleh RA. Thoracoscopic carbon dioxide laser treatment of bullous emphysema. Lancet 1991; 337:881–883.
3. Eugene J, Richard AO, Gogia HS, Cryde DS, Zeit R, Kayaleh RA. Video thoracic surgery for treatment of end-stage bullous emphysema and chronic obstructive pulmonary disease. The American Surgeon 1995; 61:934–936.
4. Little AG, Swain JA, Nino JJ, Prabhu RD, Schlachter MD, Barcia TC. Reduction pneumoplasty for emphysema: Early results. Ann Surg 1995; 222:365–374.
5. Wakabayashi A. Thoracoscopic technique for management of giant bullous lung disease. Ann Thorac Surg 1993; 56:708–712.
6. Brenner M, Kayaleh RA, Milne EN, Bella LD, Osann K, Tadir Y, Berns MW, Wilson AF. Thoracoscopic laser ablation of pulmonary bullae. Radiographic selection and treatment response. J Thoac Cardiovasc Surg 1994; 107: 883–890.
7. Keenan RJ, Landreneau RJ, Sciurba FC, Ferson PF, Holbert JM, Brown ML, Fetterman LS, Bowers CM. Unilateral thoracoscopic surgical approach for diffuse emphysema. J Thorac Cardiovasc Surg 1996; 111:308–316.
8. MacKenna RJ, Brenner M, Gelb AF, Mullin M, Singh N, Peters H, Schein MJ. A randomized, prospective trial of stapled lung reduction versus laser bullectomy for diffuse emphysema. J Thorac Cardiovasc Surg 1996; 111:317–322.
9. Hazelrigg S, Boley T, Henkle J. Thoracoscopic laser bullectomy: a prospective study with three-month results. J Thorac Cardiovasc Surg 1996; 112:319–327.
10. Brenner M , Shankel T, Berns MWA. Animal model for thoracoscopic laser of emphysematous pulmonary bullae. Lasers Surg Med 1996; 18:191–196.
11. Mitsuhashi T, Shimazaki M, Chanoki Y, Sakai T. Giant to small emphysematous bullae induced by Sephadex beads and carrageenan. Am J Patol 1986; 124:246–252.
12. Lai YL, Hildebrandt J. Respiratory mechanics in the anesthetized rat. J Appl Physiol 1978; 45(2):255–260.
13. Diamond L, O'Donnell M. Pulmonary mechanics in normal rats. J Appl Physiol 1977: 43;942–948.
14. Lai YL, Diamond L. Inhibition of porcine pancreatic elastase-induced emphysema by elgin-c. Exp Lung Res 1990; 16;547–557.
15. Weibel ER, Vidone RA. Fixation of the lung by formalin stream in a controlled state of air inflation. Am Rev Respir Dis 1961; 84:856–861.
16. Dunnill MS. Quantitative methods in the study of pulmonary pathology. Thorax 1962;17:320–328.
17. Cole PH, Wolfe WG. Mechanisms of healing in the injured lung treated with the Nd:YAG laser. Lasers Surg Med 1987; 6:574–580.
18. Sawabata N, Nezu K, Tojo T, Kawachi K, Kitamura S. Morphological examination of the lung tissue ablated with Nd:YAG laser for the treatment of bullous pulmonary disease. J Jpn Assn Thorac Surg 1995; 43:831–835.

19. Brenner M, Shankel T, Wang NS, Waite TA, Wong H, Hamilton A. CO_2 and Nd:YAG laser-induced pulmonary parenchymal lung injury in a rabbit model. Am J Respir Crit Care Med 1996; 153:1136–1140.
20. Cooper JD, Trulock EP, Triantafillou AN. Bilateral pneumonectomy (volume reduction) for chronic obstructive pulmonary disease. J Thorac Cardiovasc Surg 1995; 109: 106–119.
21. Tschernko EM, Wisser W, Klepetko W et al. The influence of lung volume reduction surgery on ventilatory mechanics in patients suffering from severe chronic obstructive pulmonary disease. Anesth Analg 1996; 83:996–1001.
22. Sciurba FC, Rogers RM, Keenan RJ. Improvement in pulmonary function and elastic recoil after lung-reduction surgery for diffuse emphysema. N Engl J Med 1996; 334: 1095–1099.
23. Gelb AF, Zamel N, McKenna RJ, Brenner M. Mechanism of short-term improvement in lung function after emphysema resection. Am J Respir Crit Care Med 1996; 154: 945–951.
24. Gelb AF, McKenna RJ, Brenner M. Contribution of lung and chest wall mechanics following emphysema resection. Chest 1996; 110:11–17.
25. O'Donnell DE, Webb KA, Bertley JC, Chau LKL, Conlan AA. Mechanisms of relief of exertional breathlessness following unilateral bullectomy and lung volume reduction surgery in emphysema. Chest 1996; 110:18–27.
26. Yaegashi H, Takahashi T. The nature, severity and distribution of obstructive airway lesions in COPD studied by morphometry and computer-assisted 3-D reconstruction. Nippon kyoubu sikkan gakkai zassi. 1990; 28(6): 828–842.
27. Lai YL. Maximal expiratory flow in the guinea pig. Lung 1988; 166:303–313.
28. Lucey EC. Maximal expiratory flow and transpulmonary pressure in the hamster. J Appl Physiol 1978; 45:840–845.